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Inheritance and Linkage Studies of Some Mutant Characters in Sugarbeets, Beta Vulgaris L.

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INHERITANCE AND LINKAGE STUDIES OF SOME MUTANT CHARACTERS IN SUGARBEETS, *BETA VULGARIS* L.

by

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ABSTRACT

The inheritance and linkage of chlorina (ch_2), virescens (vi_4), and a plantain leaf character (Pl_2Pl_3) in sugarbeets were studied. Chlorina acted as a simple recessive which confirmed work reported previously. Chlorinas from two sources were found to be identical. Virescens and plantain leaf were found to be different from those reported in the literature. Virescens acted as a simple recessive. Male sterility in the virescent material was of the a_1 Mendelian type. Plantain leaf exhibited intermediate inheritance and was found to be controlled by the action of two dominant complementary factors. Linkage tests showed vi_4 and ch_2 to be independent of each other, the a_1 gene for male sterility, and genes in the Y-R-B group. One of the plantain leaf loci, Pl_2, showed independence of the R gene.

(40 pages)

INTRODUCTION

Phenotypic variation within a species has a genetic component, the basic source of which is mutation. The occurrence of mutations in crop plants has always attracted the attention of plant breeders. Since many of these mutations are deleterious, knowledge of their transmission patterns is important to the breeder in coping with them in his breeding programs. It is also desirable to determine possible linkage associations with known marker genes. The establishment of linkage associations is basic to genetic analysis and is of both practical and basic interest to plant breeders.

This study was conducted with three mutant characters found in sugarbeets. It is hoped that knowledge of the inheritance patterns and linkage relationships of these characters will contribute to the expansion of genetic information available in sugarbeets and that it may aid in their manipulation in breeding programs.

The mutant characters chosen for this study were: virescens, chlorina, and a plantain-like leaf character. The objective of the study was to determine the inheritance of these mutants and their linkage relationships with the gene for male sterility (a_1) and other marker genes. Involved in the study was the determination of the type of male sterility found in the virescent material. It was also desirable to determine if the chlorina mutants from two sources were identical.

REVIEW OF LITERATURE

Only five of the nine possible linkage groups in sugarbeets have been proposed to date. Group I, known as the Y-R-B group, was the first linkage group established and is by far the largest. In studies of sugarbeet color types in 1936, Keller (7) demonstrated a close linkage association for the yellow pigment gene (Y), and the gene of hypocotyl color (R), and found an allelomorphic series for each gene. Abegg (1) found that B, a factor for annualness, was linked with R. These linkage relationships have been confirmed by other investigators (11, 18). Owen and Ryser (11) found that colored leaf (Cl), colored vein (cv), trout or spotted leaf (Tr), and a factor for variegated foliage (vi_1) are all linked with the Y-R-B factors. They constructed a linkage map for these seven factors. Genes for curly top disease resistance (C_1) and crinkled foliage (cr) have also shown linkage with the Y-R-B group (2, 3, 16).

The factors for monogermness (m), male sterility (a_1), black root (bl), russet root (ru), dwarfism (d), plantain leaf (pl), and a lethal lutescens (lu_2), have shown independence with regard to factors in group I (1, 11, 17, 18).

Group II includes the factor for monogermness (m), a factor for late-bolting and late-flowering (lb), and a second factor for curly top disease resistance (C_2) (14, 16). Male sterility (a_1), russet root (ru), and lutescens (lu_2) have shown independence of monogermness (9, 18).

At the present time only the gene for male sterility (a_1), which has shown independence of factors in groups I and II, has been placed

in group III (18). This gene has also shown independence with russet root (ru), dwarfism (d), and lutescens (lu_2) (18).

Lutescens (lu_2) and russet root (ru) have been proposed as marker genes in groups IV and V respectively (18). However, the independence of these two genes has not as yet been definitely established.

Chloroplast defects resulting in white, yellow, light green, or variegated foliage have been found in every species of higher and lower plants which have been carefully studied (20). Formation of the chloroplast structure requires the contribution of the genome, plasmone and plastome (19, 20). The alteration of any of these may cause abnormal development of chloroplasts.

In 1957, Savitsky (15) described the inheritance of several chlorophyll deficiencies in sugarbeets including two chlorina and three virescent types. He noted that mutations resulting in chlorina and virescent phenotypes are far more common in occurrence than those causing complete albinism. Of the known chlorophyll deficiencies in sugarbeets (2, 15), only variegated foliage (v_1) and lutescens (lu_2) have been critically studied for possible linkage associations (11, 17).

MATERIALS AND METHODS

Description of Characters

a_1 -- Abortion of pollen or Mendelian male sterility (9). Pollen abortion results from the formation of a tapetal plasmodium during anther formation (5). Sterile anthers are light green to brown in color and contain no pollen. Female floral parts develop normally.

B--Annual growth habit (1, 4, 11). Plants carrying the B factor produce seedstalks under warm temperatures and 18-24 hour photoperiods. This character is influenced strongly by environment and is also apparently dependent upon modifiers. This sensitivity often causes difficulty in classification.

ch_2-- Chlorina (figure 1). Cotyledons and all leaves are light green in color (corresponding to Cosse green in "Color Standards," by Ridgway (13)). Growth rates are greatly reduced; however, plants are viable under both greenhouse and field conditions. Savitsky (15) reported the root weight of ch_2ch_2 plants to be approximately 80% of normal.

Pl_2Pl_3 -- Plantain leaf (figure 2). Plants expressing this character have semi-parallel venation which strongly resembles the ribbed condition of Plantago major. There is a great amount of phenotypic variation with this character which causes difficulty in classifying some plants.

R--Red pigmentation (7, 11). Red coloration in sugarbeets and in some other plants is due to the presence of a flavanoid known as betanin (6). Red pigmentation is most readily observed in the hypocotyls

Figure 1. Sugarbeets showing normal (left) and chlorina (right) phenotypes.

Figure 2. A sugarbeet showing plantain-like leaf venation.

of seedlings and the crown buds of older plants. Intensity and extent of color is governed by an allelomorphic series (R, R^t, R^p, and r). R^t plants show more intense pigmentation and red stripes at the base of the petioles. R^p plants have pink petioles and hypocotyls. Plants homozygous for r are green in color.

Tr--Trout or spotted leaf (11). This mutant shows red pigmented spots on the leaf with R and yellow spots with r. Pigment is most intense in the first true leaf stage. Plants of the Tr r phenotype are difficult to classify as are older Tr plants. The intensity of pigmentation shows variation.

vi_4--Virescens (figure 3). Cotyledons of vi_4 plants are normal green; however, there is a delay in chlorophyll production in several of the early true leaves. Nearly all vi_4 plants are indistinguishable from normal plants in later stages of growth. There is considerable plant-to-plant variation in the time required to develop normal pigmentation.

Y--Yellow pigmentation and extension of color (7, 11). Y, Y^r, and y constitute an allelomorphic series and are hypostatic to R. Pigment is produced in root and foliage and is red with R and yellow to orange with r. Expression of the character is variable due to accessory and environmental factors.

Sources of Genetic Material

All genetic material for this study was acquired from Dr. J. Clair Theurer, Research Geneticist, Agricultural Research Service, U. S. Department of Agriculture, Logan, Utah.

The virescent mutant was obtained in line M415A which was homozygous for the virescent factor. This line was derived by selfing a mutant which

Figure 3. Virescens (vi_4) expression observed in the newly formed leaves of a sugarbeet seedling. Virescens is no longer expressed in the first two true leaves which have already developed normal pigmentation.

was selected in a field plot of an inbred line which had been used in a long-term seed storage experiment. Male-sterile plants were found in this line.

Two sources of chlorina were investigated. One source was line 822-1, which was homozygous for the chlorina factor. This is a self-fertile line which originated from the chlorina designated by Savitsky (15) as ch_2. Plants expressing the chlorina phenotype were also found in greenhouse plantings of lines 560035 and 560042. These self-sterile lines were derived from SLC 03 seed which was treated with thermal neutrons in 1956.

The source of the plantain leaf mutant was the line 0341, an introduction which had shown some nematode resistance. In a greenhouse planting of this line, several plants showed some plantain-like venation. This expression was variable in degree. Two plants showing the best expression were selected for use in this study. These plants were strongly self-sterile.

The gene for Mendelian male sterility (a_1) was obtained in current breeding lines 5666-4, 529 + a, and G303. The genes Y and Tr were obtained from stock sources of the marker genes, lines M7539 and M5501.

Methods of Procedure

Plants used in this study were grown during 1967 and 1968 in Logan, Utah. Plantings were made in the greenhouse and in the field. Most plants grown in the greenhouse were grown to the seedling stage in flats of soil and then transplanted to pots. In cases where very small amounts of seed were available, the seeds were germinated in a germination chamber and were then transplanted to pots. Biennial plants require special photothermal treatment to induce seedstalk initiation

(10). Induction was accomplished by placing plants, when six to eight weeks old, in a cold chamber at 4.5 C (40 F) for ten weeks. Upon return to the greenhouse conditions, seedstalk formation began.

To avoid the tedious process of hand emasculation, male-sterile or self-sterile plants were used as female parents of crosses. The inflorescences of plants to be crossed were covered with paper bags prior to anthesis. Some crosses were made by exchanging the paper bags of the parents. Other crosses were made by placing inflorescences of both parents in a single paper bag. Crosses were made when the stigmas of the female parent appeared fully developed and were near white in coloration.

Populations in which virescens was expressed were observed several times during the seedling stage to minimize the chances of misclassification. Chlorina plants were classified during the cotyledon and seedling stages. Populations containing the plantain leaf character were classified at several growth stages.

Inheritance and linkage data were obtained in F_2, F_3 and backcross generations. Chi-square (χ^2) was used to test the goodness of fit of this data with expected values. A χ^2 test for heterogeneity following the suggested procedure of Mather (8), was performed when the populations appeared to be in disagreement.

Description of Crosses

Most of the crosses referred to in the text are described here in detail.

The following crosses were made to study the inheritance of the male sterility found in association with virescens.

M7701 is the F_1 of the cross:

♀ Virescent, male sterile plant from line M415A ($vi_4vi_4RRbba_1a_1$). X ♂ Non-virescent plant ($Vi_4Vi_4rrbbA_1A_1$).

M7703 is the F_1 of the cross:

♀ Non-virescent, male-sterile plant ($Vi_4Vi_4rrbba_1a_1$). X ♂ Virescent plant found in line M415A which segregated for male sterility ($vi_4vi_4RRbbA_1a_1$).

The crosses listed below were used to study the inheritance and linkage relationships of virescens.

M7871 is the F_2 generation of the cross:

♀ Virescent, male-sterile plants ($vi_4vi_4RRbba_1a_1$). X ♂ Non-virescent plant ($Vi_4Vi_4rrbbA_1A_1$).

M7877 is the F_2 generation of the cross:

♀ Virescent, male-sterile plants ($vi_4vi_4yyrrBba_1a_1$). X ♂ Non-virescent plant ($Vi_4Vi_4YYrrbbA_1A_1$).

M7882 is the F_2 generation of the cross:

♀ Virescent, male-sterile plants ($vi_4vi_4Ch_2Ch_2RRBba_1a_1$). X ♂ Chlorina - ch_2 ($Vi_4Vi_4ch_2ch_2rrbbA_1A_1$).

M7737 is the BC_1 generation of the cross:

♀ Virescent, male-sterile plants ($vi_4vi_4rrBba_1a_1$). X ♂ F_1 plants from line M7703 ($Vi_4vi_4RrbbA_1a_1$).

The crosses listed below were used to study the inheritance of the chlorinas from the two sources and their linkage relationships.

M7862 is the F_2 generation of the cross:

♀ Normal, male-sterile plants ($Ch_2Ch_2rrbba_1a_1$). X ♂ Chlorina - ch_2 ($ch_2ch_2rrbbA_1A_1$).

M7873 is the F_2 generation of the cross:

♀ Normal, male sterile plants ($Ch_2Ch_2rrbba_1a_1$). X ♂ Chlorina from irradiated SLC 03 ($ch_2ch_2rrBBA_1A_1$).

M7743 is the F_1 generation of the cross:

♀ Chlorina - ch_2 (ch_2ch_2rrbb). X ♂ Chlorina from irradiated SLC 03 (ch_2ch_2rrbb).

M7744 is the F_1 generation of the cross:

♀ Chlorina from irradiated SLC 03 (ch_2ch_2rrbb). X ♂ Chlorina - ch_2 (ch_2ch_2rrbb).

M7882 is the F_2 generation of the cross:

♀ Virescent, male-sterile plants ($vi_4vi_4Ch_2Ch_2RRBba_1a_1$). X ♂ Chlorina - ch_2 ($Vi_4Vi_4ch_2ch_2rrbbA_1A_1$).

The following cross was made to determine the inheritance of the plantain leaf character.

♀ Male-sterile plant with normal venation and green hypocotyl from line 5666-4. X ♂ Plantain leaf mutant with red hypocotyl from line 0341.

The F_1 generation of the cross is M7702. Populations designated M7860 and M7878 are the F_2 and F_3 generations of this cross.

RESULTS AND DISCUSSION

Determination of Inheritance

Male sterility found in association with virescens

Crosses between male-sterile plants found in line M415A and plants true breeding for fertility produced no male-sterile plants in the F_1 generation (Table 1). In the F_2 generation, however, both male-sterile and fertile plants were observed. No partial-fertile phenotypes were observed. These observations would imply that the sterility is nuclear rather than cytoplasmic.

Table 1 shows the segregation of male sterility in the F_1 generation of a cross between a_1a_1 and a selection from line M415A. This segregation fits closely to a 1:1 backcross ratio. A backcross ratio indicates that the male parent of the cross was heterozygous for the a_1 gene. These observations indicate that the male sterility found in the virescent line M415A is dependent upon the a_1 gene.

Virescens -- vi_4

Table 2 presents data for the F_1 and BC_1 generations of crosses between virescent and true breeding normal plants. None of the F_1 plants grown showed virescent expression. Backcross data in Table 2 and F_2 data in Table 3 show monohybrid segregation ratios. These observations indicate that the inheritance of virescens is controlled by a simple recessive gene.

Population M7882 in Table 3 shows obvious deviation from the expected values. This population accounts for most of the deviation in

Table 1. Segregation for the male sterility found associated with virescens.

Families	Fertile (obs.)	Fertile (exp.)	Male Sterile (obs.)	Male Sterile (exp.)	χ^2	P*
[♀ Male sterile associated with virescens X ♂ Pollinator of the A_1A_1 type]						
F_1						
2 families	41	41	0	0		
F_2						
M7871-1	91	84.8	22	28.2	1.844	.10-.20
M7871-2	83	85.5	31	28.5	0.292	.50-.70
M7871-3	76	77.2	27	25.8	0.081	.70-.80
Total	250	247.5	80	82.5	0.101	.70-.80
[♀ a_1a_1 male sterile X ♂ virescens from segregating population]						
F_1						
M7703	43	40.5	38	40.5	0.309	.50-.70

* Expected on the basis of a 3:1 ratio in the F_2 and a 1:1 ratio in the F_1.

Table 2. The inheritance of vi_4 observed in the F_1 and backcross generations of crosses with normal plants.

Families	No. of Plant	Normal	Virescent	χ^2	P
F_1					
♀ vi_4vi_4 X ♂ Vi_4Vi_4					
5 families	112	112	0		
♀ Vi_4Vi_4 X ♂ vi_4vi_4					
2 families	53	53	0		
Total	165	165	0		
BC_1 M7737	273	149	124	2.289	.10-.20
(expected on the basis of a 1:1 ratio)		136.5	136.5		

Table 3. The inheritance of vi_4 observed in the F_2 generation of crosses with non-virescent lines.

Families	Normal (obs.)	Normal (exp.)	Virescens (obs.)	Virescens (exp.)	χ^2	P*
M7871	315	321.8	114	107.2	0.566	.30-.50
M7831	211	210.0	69	70.0	0.019	.50-.70
M7877	179	180.8	62	60.2	0.068	.70-.80
M7882	451	398.2	80	132.8	27.948	<.001
Total	1156	1110.8	325	370.2	7.374	.001-.01

* Expected on the basis of a 3:1 ratio.

Partition of χ^2

Item	χ^2	d.f.	P	Mean square
Deviation	7.374	1	.001-.01	7.374
Heterogeneity	21.227	3	<.001	7.076
Total	28.601	4		

Partition of χ^2 when the population M7882 is deleted

Item	χ^2	d.f.	P	Mean square
Deviation	0.316	1	.50-.70	0.316
Heterogeneity	0.337	2	.80-.90	0.168
Total	0.653	3		

the total χ^2. Partitioning of the χ^2 indicates a probability of less than .001 that the families are in agreement. Note, however, that the χ^2 test for heterogeneity does not show significance when M7882 is deleted.

The reason for the deviation of population M7882 is not understood. This is the F_2 generation of a cross between virescens and chlorina -ch_2. Difficulty in the identification of double recessive plants could be expected to account for some of the deviation. It should be noted, however, that the number of plants in the other virescent class was also far below the expected amount. The close adherence of genes R and ch_2 to a monohybrid ratio would seem to indicate that contamination of pollen was not a factor causing the deviation. It would seem that this apparent deviation resulted from a change in the penetrance of the mutant.

Savitsky (15) described three virescent types vi_1, vi_2, and vi_3. Each of these types differed in productiveness and degree of delay in chlorophyll production. The seedlings of these three virescens were described as being golden-yellow in color, and they developed chlorophyll in the later leaves. These virescens demonstrated sufficient delay in chlorophyll production to cause lethality under field conditions and general unproductiveness in the greenhouse. The virescent plants obtained from line M415A were not golden-yellow in color in the seedling stage nor did they demonstrate a delay in chlorophyll production sufficient to cause death under field conditions. Plants of this virescent type were near normal in size and productivity. The virescent type found in line M415A has been designated vi_4. The virescent plants observed showed a delay in chlorophyll production which was most pronounced in the first true leaves. The color of the virescent leaves observed varied, ranging from near white to light

green. The delay in pigmentation became less apparent as subsequent leaves were formed until the plants became indistinguishable from normal. There was considerable variation observed in the time required for normal pigmentation to develop. Most virescent plants appeared normal after the formation of the first few sets of true leaves. However, in a field planting in 1968, a plant nearing maturity was observed which demonstrated very pronounced delay of pigmentation in the newly formed leaves. Such variation in expression caused difficulty in the classification of some plants.

Chlorina--ch_2

Reciprocal crosses between the two sources of chlorina, ch_2 and that derived from irradiated SLC 03 seed, produced only chlorina plants (Table 4). It was concluded that the chlorinas from the two sources were identical. This chlorina will be referred to as ch_2 consistent with the designation of Savitsky (15).

As shown in Table 5, progeny of reciprocal crosses involving normal and chlorina plants were green in the F_1 generation. F_2 populations (Table 5) demonstrated monohybrid segregation. These data confirm the conclusion of Savitsky (15), that ch_2 acts as a simple recessive.

The population M7873 showed significance at the 5% level of probability and also caused the χ^2 test of heterogeneity to be significant (Table 5). The deviation demonstrated in this population could have been a chance occurrence or it might have been the result of the radiation treatment of SLC 03 from which this population was derived.

Table 4. The F_1 generation of reciprocal crosses between ch_2 and the chlorina derived from irradiated SLC 03 seed.

	Normal	Chlorina
♀ ch_2ch_2X ♂ SLC 03 source		
2 families	0	8
♀ SLC 03 source X ♂ ch_2ch_2		
2 families	0	83
Total	0	91

Table 5. The inheritance of the ch_2 gene observed in crosses with normal green plants.

Families	Green (obs)	Green (exp.)	Chlorina (obs.)	Chlorina (exp.)	χ^2	P*
F_1						
♀ chlorina X ♂ normal						
3 families	5	5	0	0		
♀ normal X ♂ chlorina						
3 families	72	72	0	0		
Total	77	77	0	0		
F_2						
Chlorina - ch_2						
M7882	408	398.2	123	132.8	0.955	.30-.50
M7862	306	317.2	117	105.8	1.596	.20-.30
Chlorina from irradiated SLC 03						
M7873	106	95.2	21	31.8	4.853	.02-.05
Total	820	810.8	261	270.2	0.422	.50-.70

* Expected on the basis of a 3:1 ratio.

Partition of χ^2

Item	χ^2	d.f.	P	Mean square
Deviation	0.422	1	.50-.70	0.422
Heterogeneity	6.982	2	.02-.05	3.491
Total	7.304	3		

Plantain leaf--Pl_2Pl_3

The progeny of a cross between a male-sterile plant from line 5666-4, a line which was true breeding for normal venation, and a plantain leaf mutant from line 0341 were observed in the F_1, F_2, and F_3 generations. Plantain leaf expression was observed in the F_1 generation; however, the plants did not express the character until they were several weeks old. Table 6 shows a segregation ratio of 1 plantain : 3 normal in a population of 36 plants. The F_1 plants showing the best plantain leaf expression were selfed to produce the F_2 generation. The F_2 generation produced a gradation of character expression. It is noted that there was a deficiency of plantain leaf mutants in both F_2 lines (Table 6), when the data were fit to a 9:7 ratio. The population M7860-16 was deficient enough to cause significance at the 5% level of probability. A portion of this deficiency was perhaps due to misclassification of plants with very poor character expression.

The F_1 and F_2 data of this cross suggest that this plantain leaf mutant has dominant expression and is controlled by the action of two complementary genes. Plantain leaf expression is not expected therefore, unless one allele appears dominant at each locus. This is a different plantain leaf mutation than the simple recessive described in previous studies and designated pl (1, 11). The complementary factors conditioning the plantain leaf character were designated Pl_2 and Pl_3.

It is noted that segregation in the F_1 generation, fits closely to a 1:3 ratio (Table 6). This ratio would imply that the mutant parent was homozygous dominant at one locus and heterozygous at the other; a 1:1 ratio would be expected. A 1:3 ratio also implies that the normal parent of the cross does not carry the dominant allele at either locus. This is mentioned because it must be remembered that a

Table 6. The inheritance of the plantain leaf mutant observed in the cross ♀ $a_1a_1pl_2pl_2pl_3pl_3$ X ♂ $A_1A_1Pl_2pl_2Pl_3pl_3$.

Families	Plantain leaf (obs.)	Plantain leaf (exp.)	Normal venation (obs.)	Normal venation (exp.)	χ^2	P*
F_1 M7702	10	9	26	27	0.148	.70-.80
F_2 M7860-3	118	125.4	105	97.6	1.008	.30-.50
M7860-16	78	91.7	85	71.3	4.670	.02-.05
Total	196	217.1	190	168.9	4.698	.02-.05
F_3 M7878-1	112	106.5	30	35.5	1.136	.20-.30
M7878-10	17	17.2	6	5.8	0.014	>.90
Total	129	123.7	36	41.3	0.891	.30-.50

* Expected on the basis of a 1:3 ratio in the F_1, a 9:7 ratio in the F_2, and a 3:1 ratio in the F_3.

Partition of χ^2

Item	χ^2	d.f.	P	Mean Square
F_2				
Deviation	4.698	1	.02-.05	4.698
Heterogeneity	0.980	1	.30-.50	0.980
Total	5.678	2		
F_3				
Deviation	0.891	1	.30-.50	0.891
Heterogeneity	0.259	1	.50-.70	0.259
Total	1.150	2		

line may breed true to a normal phenotype and could still be homozygous for the dominant allele at one locus. The presence of a dominant allele in such a plant would only become apparent with the interaction of its complement. The presence of a dominant allele at either locus in this normal beet would raise the proportion of plantain leaf mutants produced in the progeny.

If a plantain leaf mutant not fully homozygous dominant is selfed, the progeny are expected to follow either of two segregation ratios. In a case where a plant which is heterozygous at each locus is selfed, the progeny are expected to segregate in a 9:7 ratio. The character should also be expressed if the plant is heterozygous at one locus and homozygous dominant at the other. In such a case a 3:1, or monohybrid ratio, is expected. A 3:1 ratio was obtained in the F_3 as shown in Table 6, implying that one gene pair was segregating in these two populations.

There was a gradation of phenotypic expression noted with this mutant character. It was noted at the beginning of this section that the F_1 progeny of the cross 5666-4 X 0341 did not express the plantain leaf character in the early stages of growth. Some of the ten plants which were eventually classified as plantain mutants began to show definite character expression when they were approximately six weeks old. Until this time only normal venation was observed. The manifestation of the character became stronger with later stages of growth. As seedstalk initiaion began, each of the ten mutants showed expression in a majority of the leaves. Only one plant was observed, however, that showed expression in each leaf of the rosette and each leaf produced on the seedstalk.

More variation was noted in the F_2 and F_3 generations of this cross. In these two generations, some plants were observed which showed plantain-like expression in every leaf formed from the seedling stage on. Many other plants developed the expression similar to the F_1 plants described above.

In this discussion, the phenotypic expression will be referred to as good or as intermediate. Good expression refers to the type of expression in which each leaf formed by the plant, beginning with the first true-leaf stage, showed plantain-like venation. This type of expression was noted in the mutant parent from line 0341 and in the F_2 and F_3 generations. The intermediate classification covers a range of phenotypes. One of the intermediate types was that observed in the F_1 where expression was delayed. This classification also includes the plants which showed expression of the character in all but one or two leaves. Some intermediate plants showed mutant expression in only one or two leaves or had only a tendency toward parallel venation.

Populations M7878-1 and M7878-10 were derived from selfing F_2 plants which were classified as intermediate in character expression. These two populations produced plants with good, intermediate, and non-plantain expression approaching a 1:2:1 ratio (Table 7), which is a variation of the 3:1 ratio mentioned in the above discussion. A 1:2:1 ratio implies the action of incomplete dominance. It is obvious, however, that the same locus may be heterozygous in both of these populations. A difference of gene expressivity may exist with the other locus. Thus, it cannot be concluded from these data that an incomplete dominance effect should be expected in all cases where one locus is found to be heterozygous.

Table 7. Variation of plantain expression observed in the F_3 generation of the cross ♀ $a_1a_1pl_2pl_2pl_3pl_3$ X ♂ $A_1A_1Pl_2pl_2Pl_3Pl_3$.

Expression of parent	Families	Mutant expression: Good	Intermediate	None	
good	M7878-3	19	1	0	
	M7878-8	15	63	1	χ^2 = 32.924
	(exp.)*	19.8	39.5	19.8	P = <.001
inter-mediate	M7878-1	30	82	30	
	M7878-10	3	14	6	
	Total	33	96	36	χ^2 = 4.527
	(exp.)*	41.2	82.5	41.2	P = .10-.20

* Expected on the basis of a 1:2:1 ratio.

Populations M7878-3 and M7878-8 were obtained through selfing F_2 plants with good expression. The populations M7878-3 produced plants with good expression with the exception of one intermediate (Table 7). This information indicates a homozygous dominant condition at both loci. The one plant classified as intermediate may have been identical to the others in genotype but for some reason showed poor character expression, or it may have been the result of contamination of pollen.

The populations M7878-3, M7878-1 and M7878-10 suggest a cumulative type gene effect. Population M7878-8, however, deviated strongly from this pattern. The parental genotype of this population remains in question. If the parent was heterozygous at one or both loci, the progeny would be expected to fit a 3:1 or a 9:7 ratio. Obviously M7878-8 does not fit either of these ratios (Table 7). If the parent was homozygous dominant at both loci, and character expression was dependent upon a cumulative effect, only plants having good expression would be expected in the progeny. This population, however, produced a rather high number of plants with intermediate expression. Thus, the expression observed in this case cannot be adequately explained on the basis of the present data.

Linkage Relationships

The χ^2 tests for deviations in Mendelian ratios and linkage relationships of the mutants under discussion are given in Table 8. The designations F_2R, BC_1C, etc. under the heading "linkage phase", refer to the generation in question and coupling or repulsion of the genes in the cross. For example: families following an F_2R designation are in the F_2 generation and the genes entered the cross in repulsion, families following a BC_1C designation are in the first backcross

Table 8. χ^2 tests for deviations in Mendelian ratios and linkages of vi_4, ch_2, Pl_2 with a_1 and genes of the Y-R-B group.

Genes (XY)	Linkage Phase	No. of families	No. of plants	Phenotypic Segregation XY	Xy	xY	xy	χ^2_X	χ^2_Y	χ^2_L
Y B	F_2R	4	256	122	64	62	8	0.750	1.333	14.694**
R B	F_2R	3	429	233	93	100	3	0.225	1.573	26.356**
R a_1	F_2R	3	330	177	56	73	24	3.308	0.101	0.121
	BC_1C	1	98	27	20	25	26	0.163	0.367	0.653
vi_4Y	F_2C	3	241	132	47	43	19	0.068	0.732	0.502
vi_4R	F_2R	4	717	405	130	146	36	0.056	1.306	1.519
	BC_1R	2	273	66	83	66	58	2.289	0.297	2.289
vi_4B	F_2C	3	429	246	69	84	30	0.566	0.846	0.842
	BC_1C	2	104	22	33	22	27	0.346	2.462	0.346
	F_2R	3	240	135	43	38	24	0.089	1.089	5.400*
vi_4a_1	F_2C	3	330	191	56	59	24	0.004	0.101	1.294

Table 8. Continued

Genes (XY)	Linkage Phase	No. of Families	No. of Plants	Phenotypic Segregation XY	Xy	xY	xy	χ^2_X	χ^2_Y	χ^2_L
	BC_1R	1	98	28	24	24	22	0.367	0.367	0.041
vi_4ch_2	F_2R	3	531	343	108	65	15	27.955**	0.955	0.352
ch_2R	F_2R	3	531	323	85	94	29	0.955	3.531	0.462
ch_2a_1	F_2R	1	231	117	36	45	15	1.141	0.127	0.042
Pl_2R	F_3C	1	143	86	27	24	6	1.233	0.282	0.131

* A χ^2 value of 3.841 is significant at the 5% level.
** A χ^2 value of 6.635 is significant at the 1% level.

generation and they entered the cross in the coupling phase.

The total χ^2 as such, is not listed, but has been partitioned into the χ^2_X, χ^2_Y and χ^2_L components as suggested by Mather (8). The χ^2_X and χ^2_Y refer to the fit of the genes designated X and Y to 3:1 ratios. The χ^2_L refers to the interaction or linkage of the two genes in question. These χ^2 values have one degree of freedom.

Virescens--vi_4

Linkage data in Table 8 show that vi_4 is independent of the genes Y, R, and B which are marker genes of linkage group I. The significance noted in χ^2_L of vi_4B is accounted for by the deviation in one population. The population M7877-1 shows significance at the 1% level of probability (Table 9). This family is however included in the data with the other F_2R lines because the heterogeneity χ^2 value was not significant. The reason for the significance noted in M7877-1 is uncertain. It is possible that a translocation of genetic material might have occurred in the parent which would in effect establish linkage in a portion of the chromosomes. Sufficient seed was not available to investigate this possibility. Considering that independence was shown in the other families of crosses between vi_4 and the group I marker genes, the importance of the significance noted in M7877-1 is doubtful with regard to the final analysis.

Crosses were also made between vi_4vi_4 and TrTr, the trout or spotted leaf mutant which is closely linked with R in the Y-R-B group. However, no linkage data are presented for vi_4 Tr. The heavy production of pigment in the first true leaves, characteristic of trout leaf, tends to cover the expression of vi_4. As a result, only a portion of the trout leaf mutants expressing virescens could be identified.

Table 9. χ^2 tests for linkage in individual families of the cross ♀ vi_4vi_4Bb X ♂ Vi_4Vi_4bb (F_2R).

Families	No. of plants	Vi_4B	Vi_4b	vi_4B	vi_4b	χ^2_L	P
M7877-1	107	60	19	14	14	7.860	.001-.01
M7877-2*	44	25	7	9	3	0.031	.80-.90
M7877-4	89	50	17	15	7	0.361	.50-.70
Total	240	135	43	38	24	5.400	.02-.05

Partition of χ^2_L

Item	χ^2	d.f.	P	Mean square
Deviation	5.400	1	.02-.05	5.400
Heterogeneity	2.852	2	.20-.30	1.426
Total	8.252	3		

Partition of χ^2_L when the population M7877-1 is deleted

Item	χ^2	d.f.	P	Mean square
Deviation	0.368	1	.50-.70	0.368
Heterogeneity	0.024	1	.80-.90	0.024
Total	0.392	2		

* Yates correction for continuity was applied because of the low expected frequency in the double recessive class.

Virescens (vi_4) also showed independence of the a_1 and ch_2 genes.

Chlorina--ch_2

The linkage data in Table 8 show that ch_2 is independent of the genes R, a_1, and vi_4. The significance of the χ^2 for virescens is accounted for by the deviation described in the discussion of virescent inheritance.

Plantain leaf--Pl_2Pl_3

Data in Table 8 show R to be independent of the locus which was heterozygous in the F_3 population M7878-1. This locus will be designated Pl_2.

Y-R-B

The linkage relationships of Y with B, and R with B were confirmed. The data also confirm the independence of R and a_1.

SUMMARY

Inheritance and linkage relationships were studied for three mutant characters in sugarbeets: virescens, chlorina, and plantain leaf. The virescens was a different type than that described previously and it has been designated vi_4. Virescens was observed to act as a simple recessive. Chlorinas from two sources were found to be controlled by the same gene. The inheritance of chlorina as a simple recessive was confirmed. Plantain leaf was found to be controlled by two dominant complementary genes which were designated Pl_2 and Pl_3. This is a different mutant than has been described by other researchers.

Linkage data show the gene vi_4 to be independent of Y, R, B, a_1 and ch_2. The gene ch_2 exhibited independence of a_1, vi_4, and R. One of the complementary factors for plantain leaf, Pl_2, showed independence of R.

Considerable variation was observed in the expression of vi_4 and Pl_2Pl_3. There was variation noted in the amount of time required to develop normal pigmentation in the virescent plants observed and also in the coloration of virescent leaves. Intermediate inheritance was observed in the plantain leaf mutant. Incomplete dominance at one of the loci was suggested as one of the causes of the variation in Pl_2Pl_3.

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